Award Number: W81XWH-14-1-0384

TITLE:
Identification of prostate cancer-specific circular RNAs

PRINCIPAL INVESTIGATOR:
Yin-Yuan Mo

CONTRACTING ORGANIZATION:
University of Mississippi Medical Center
Jackson, MS 39216

REPORT DATE: December 2016

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
December 2016	Final	09/15/2014 to 09/14/2016

4. TITLE AND SUBTITLE
Identification of prostate cancer-specific circular RNAs

5a. CONTRACT NUMBER
W81XWH-14-1-0384

5b. GRANT NUMBER
PC131272

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Yin-Yuan Mo

E-Mail: ymo@umc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Mississippi Medical Center
2500 State Street, G652
Jackson, MS 39216

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The major goal of this application is to determine whether newly identified circular RNAs can serve as novel biomarkers for prostate cancer diagnosis and prognosis. There are three specific aims. First, we will determine whether prostate cancer cells display different patterns of circular RNAs from those of normal tissue. Then, we will determine whether differential expression of circular RNAs can also be detected in cell culture models because we can easily manipulate the levels of circular RNAs in cell culture so that we are able to study their functions. Finally, we will determine whether circular RNAs are differentially expressed in blood/serum samples from healthy individuals and prostate cancer patients.

15. SUBJECT TERMS
microRNA, lncRNA, biomarker

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	15	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Introduction

Circular RNAs (circRNAs) are a new type of long non-coding RNAs (lncRNAs). Like classic lncRNAs, circRNAs do not code for protein. However, while classic lncRNAs are linear, circRNAs are circular often through back splicing. Moreover, they often have regulatory functions. For example, circRNAs can serve as endogenous microRNA sponges to neutralize the microRNA function. However, it is not clear whether prostate cancer can exploit this mechanism for its own advantage. We would like to determine whether circRNAs are aberrantly present in prostate cancer compared to normal tissue. Identification of such dysregulated circRNAs would lay a foundation for us to explore their role in prostate cancer and to identify novel prostate cancer biomarkers or therapeutic targets. We hypothesize that prostate cancer may exploit this mechanism for its own advantage and thus prostate cancer may display a very different circRNA pattern from normal prostate tissue. Therefore, the major goal of this application is to determine whether newly identified circular RNAs can serve as novel biomarkers for prostate cancer diagnosis and prognosis.

Body

CircRNAs are aberrantly expressed in prostate cancer. As newly discovered molecules, circRNAs are poorly characterized. Little is known whether they are dysregulated in prostate cancer. Thus, our first step was to characterize these new molecules by profiling. Results indicate that a number of circRNAs are either upregulated (Table 1) or downregulated (Table 2) in tumor tissue as compared to normal tissue.

For example, 15 upregulated circRNAs have over 1.5-fold increase in tumor vs normal with p value <0.05. The expression level of hsa_circRNA_104595 was a 2.7-fold higher in tumor than in normal. To better illustrate how the circular form is formed, we provide the sequence for hsa_circRNA_002143, as shown in Fig. 1 as an example. The top part is the actual sequence and the bottom part is when a circle is formed. Two ends at the junction were highlighted by red and blue, respectively.

On the other hand, 18 circRNAs were at least 2-fold decrease in tumor vs normal. For example, hsa_circRNA_002143 was detected about a 3-fold downregulation in tumor as compared to normal (Table 2). We also provide schematic illustration of hsa_circRNA_002143, as shown in Fig. 2.

CircRNAs are derived from various sources. The origin of these circRNAs varies, ranging from intronic, intragenic to exonic. Intronic circRNAs originate from introns; intragenic circRNAs originate from the regions between two separate genes; and exonic circRNAs originate from exons. Furthermore, these exons can be for coding genes or non-coding genes.

CircRNAs can potentially target microRNAs. One of potential functions for circRNAs is the capability to serve as sponges to neutralize the endogenous microRNAs. In this regard, all of these circRNAs had the potential to target more than one microRNA. We listed one for each in Table 1 and Table 2. This suggests that aberrant expression of these circRNAs may affect the levels of these microRNAs, thus, contributing to prostate

tumorigenesis. For example, there are two binding sites for miR-412-3p and one binding site for miR-363-5p in hsa_circRNA_104595 (Fig. 3). On the other side, there are over 20 binding sites for miR-663a in hsa_circRNA_002143 probably because hsa_circRNA_002143 is much larger than hsa_circRNA_104595. We just listed five of them (Fig. 4). To determine whether overexpression of circRNAs can affect microRNA expression, we chose hsa_circRNA_002143. As shown in Fig. 5 A, miR-412-3p was downregulated in the cells with overexpression of hsa_circRNA_104595 as compared to vector control. For miR-363-5p, we only detected a slight downregulation in hsa_circRNA_104595 cells. Moreover, hsa_circRNA_104595 promoted cell growth (Fig. 5B), suggesting that it plays an oncogenic role.

hsa_circRNA_104595 is upregulated in serum samples of prostate patients. To determine whether circRNAs are deregulated in serum samples, we chose both hsa_circRNA_104595 and hsa_circRNA_002143. We detected upregulation of hsa_circRNA_104595 in serum samples of breast patients (Fig. 6). In contrast, we were not able to deregulation of hsa_circRNA_002143

Key Research Accomplishments

- We identified 15 upregulated and 18 downregulated circRNAs from prostate cancer cells through profiling.
- All of these circRNAs carry microRNA binding sites, through which they may regulate the level of endogenous microRNAs.

- We found that hsa_circRNA_104595 can negatively regulate miR-412-3p and promote tumor cell growth.
- Finally, we showed that hsa_circRNA_104595 is upregulated in serum samples of breast cancer patients. Thus, hsa_circRNA_104595 may serve as a biomarker for prostate cancer.

Reportable Outcomes

"Hsa_circRNA_104595 as a potential biomarker for prostate cancer" in preparation.

Conclusions

Microarray profiling has identified 15 upregulated and 18 downregulated circRNAs from prostate cancer cells. Ectopic expression of hsa_circRNA_104595 downregulates expression of miR-412-3p and promotes tumor cell growth. Therefore, further characterization of circRNAs in prostate cancer will help identify novel circRNA-based biomarkers.

Table 1, Upregulation of circular RNAs in tumors

Name	Tumor/normal	P-value	circRNA_type	Potential miR binding
hsa_circRNA_104595	2.717446	0.003168	exonic	hsa-miR-412-3p
hsa_circRNA_100790	2.0369969	0.015794	exonic	hsa-miR-20b-3p
hsa_circRNA_104927	1.9599525	0.044262	exonic	hsa-miR-500a-3p
hsa_circRNA_102605	1.9344202	0.002482	exonic	hsa-miR-486-3p
hsa_circRNA_000956	1.926276	0.022407	antisense	hsa-miR-765
hsa_circRNA_000554	1.7791893	0.009459	intronic	hsa-miR-153-5p
hsa_circRNA_100438	1.6898966	0.014578	exonic	hsa-miR-383-3p
hsa_circRNA_101175	1.685527	0.033942	exonic	hsa-miR-374a-3p
hsa_circRNA_103975	1.6771255	0.042994	exonic	hsa-miR-493-5p
hsa_circRNA_103950	1.6427088	0.00164	exonic	hsa-miR-143-5p
hsa_circRNA_102889	1.6353354	0.030357	exonic	hsa-miR-9-5p
hsa_circRNA_102545	1.5942883	0.02303	exonic	hsa-miR-573
hsa_circRNA_103417	1.55695	0.011534	exonic	hsa-miR-597-3p
hsa_circRNA_100213	1.5444917	0.041263	exonic	hsa-miR-345-5p
hsa_circRNA_105037	1.5315459	0.000508	exonic	hsa-miR-197-3p

Table 2, Downregulation of circular RNAs in tumors

Name	Tumor/normal	P-value	circRNA_type	Potential miR binding
hsa_circRNA_002143	0.373893479	0.002055	intragenic	hsa-miR-663a
hsa_circRNA_100477	0.373893479	0.004311	exonic	hsa-miR-134-5p
hsa_circRNA_101164	0.373893479	0.003234	exonic	hsa-miR-103a-2-5p
hsa_circRNA_101615	0.373893479	0.004198	exonic	hsa-miR-197-3p
hsa_circRNA_000911	0.446551827	0.01212	intronic	hsa-miR-449c-3p
hsa_circRNA_104084	0.446551827	0.012523	exonic	hsa-miR-506-3p
hsa_circRNA_000780	0.461434466	0.018459	intronic	hsa-miR-651-3p
hsa_circRNA_102701	0.461434466	0.015917	exonic	hsa-miR-369-3p
hsa_circRNA_104121	0.461434466	0.019856	exonic	hsa-miR-203a-3p
hsa_circRNA_104930	0.461434466	0.019407	exonic	hsa-miR-762
hsa_circRNA_104204	0.478932053	0.03059	exonic	hsa-miR-619-5p
hsa_circRNA_101213	0.485389422	0.033127	exonic	hsa-miR-431-3p
hsa_circRNA_104666	0.486375944	0.034278	exonic	hsa-miR-1468-5p
hsa_circRNA_100750	0.496412771	0.037023	exonic	hsa-miR-1301-3p
hsa_circRNA_000881	0.499530875	0.044016	intronic	hsa-miR-557
hsa_circRNA_102445	0.499530875	0.042097	exonic	hsa-miR-644a
hsa_circRNA_103134	0.499530875	0.044068	exonic	hsa-miR-644a
hsa_circRNA_101336	0.501698841	0.04572	exonic	hsa-miR-320b

CCCAGCCCTGGGGAGCCCCTGTGGAAGTGGAGTCCTTCCTGGTCCACCCCGGTGACCTGCT
GCAGCTTCGCTGTCGGCTGCGGGACGATGTGCAGAGCATCAACTGGCTGCGGGACGGGGTG
CAGCTGGCGGAAAGCAACCGCACCCGCATCACAGGGGAGGAGGTGGAGGTGCAGGACTCCG
TGCCCGCAGACTCCGGCCTCTATGCTTGCGTAACCAGCAGCCCCTCGGGCAGTGACACCAC
CTACTTCTCCGTCAATGTTTCAG

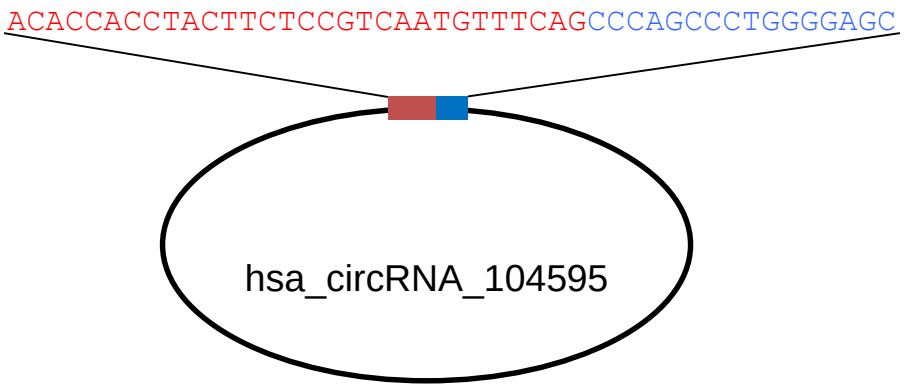


Fig. 1 DNA sequence of hsa_circRNA_104595. The junction of two ends is highlighted by red and blue, respectively.

AGACAAGGTAGCTCCATAATGGTCAGGCTGGTCTAGAACACCCAACCTGAGGCGTACCACCCAACTTGACCACCCAAAG
TGCTGAGATTAAAGGCGTGAGCTCCGCGTCTGGCCATAACATCTTATCCTATAGAAGCCCAGAGAGGTTAGGCGTCATC
TCACGTGTCGAGGTGATCTCGAACTTTTAGGCTCCAGAGATCCTCCCGCATCGGCCTCCCGGAGTGCTGTGATGACACG
CGTGGGCACGACGGAGTTTCACTCTTGTCGCCCAGGGTGGAGTACGATGGCGGCTCTCGGCTCACCGCACCCTCCGCCT
CCCAGGTTCAAGTGATTCTCCTGCCTCAGCCTTCCCGAGTAGCTGGAATGACAGAGATGAGCCATCGTGCCCGGCTAAT
TTTTCTATTTTTACTACAGATGGGGTTTCTCCATCTTGGTCAGGCTGGTCTTCAACTTCCGACCGTTGGAGAATCTTAA
CTTTCTTGGTGGTGGTTGTTTTCCTTTTTCTTTTTTTTCTTTTCTTTTCTTTCCTTCTCCTCCCCCCCCACCCCCCCTT
GTCGTCGTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCCTCTTTCATTTCTTTCAGCTGGGCT
CTCCTACGTGTGTTGCTCTGTTGCTCACGCTGGTCTCAAACTCCTGGCCTTGACGCTTCTCCCGTCACATCCGCCGTCT
GGTTGTTGAAATGAGCATCTCTCGTAAAATGGAAAAGATGAAAGAAATAAACACGAAGACGGAAAGCACGGTGTGAACG
TTTCTCTTGCCGTCTCCCGGGGTGTACCTTGGACCCGGAAACACGGAGGGAGCTTGGCTGAGTGGGTTTTCGGTGCCGA
AACCTCCCGAGGGCCTCCTTCCCTCTCCCCCTTGTCCCCGCTTCTCCCCCAGCCGAGGCTCCCACCGCCGCCCTGGCAT
TTTCCATAGGAGAGGTATGGGAGAGGACTGACACGCCTTCCAGATCTATATCCTGCCGGACGTCTCTGGCTCGGCGTGC
CCCACCGGCTACCTGCCACCTTCCAGGGAGCTCTGAGGCGGATGCGACCCCCACCCCCCCGTCACGTCCCGCTACCCTC
CCCCGGCTGGCCTTTGCCGGGCGACCCCAGGGGAACCGCGTTGATGCTGCCTTCGGATCCTCCGGCGAAGACTTCCACC
GGATGCCCCGGGTGGGCCGGTTGGGATCAGACTGGACCACCCCGGACCGTGCTGTTCTTGGGGCACACAGATGAGACGC
ACGAGAGGGAGAAACAGCTCAATAGATACCGCTGACCTTCATTTGTGGAATCCTCAGTCATCGACACACAAGACAG

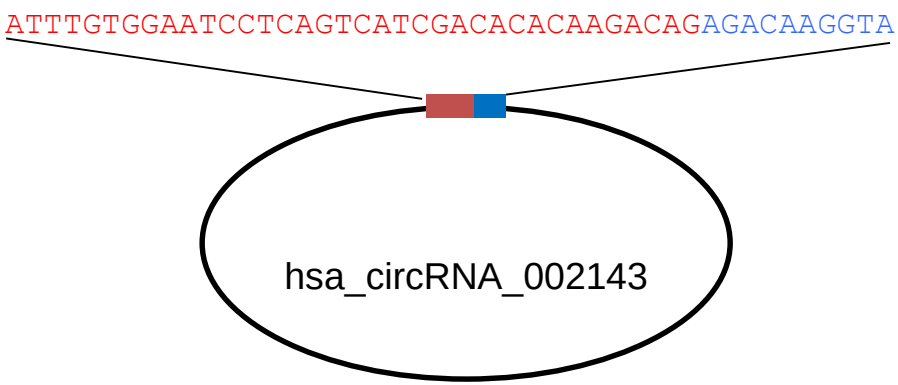
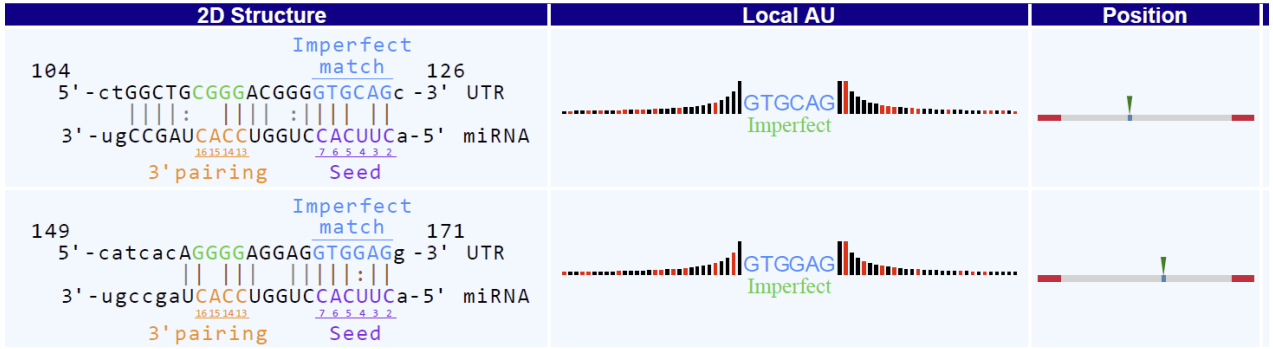


Fig. 2 DNA sequence ofhsa_circRNA_002143. The junction of two ends is highlighted by red and blue, respectively.

MicroRNA binding sites in hsa_circRNA_104595

hsa-miR-412-3p



hsa-miR-363-5p

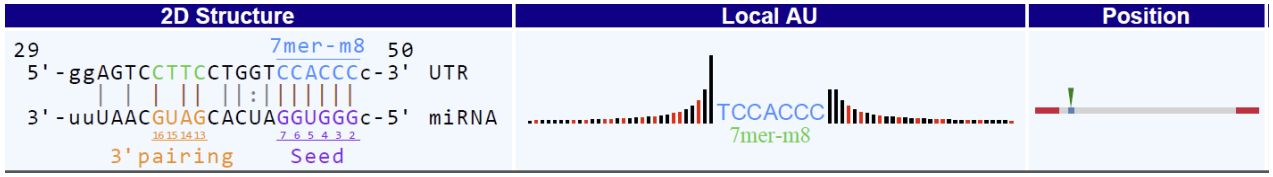


Fig. 3 Binding sites in hsa_circRNA_104595 for hsa-miR-412-3p and has-miR-363-5p.

MicroRNA binding sites in hsa_circRNA_002143

hsa-miR-663a

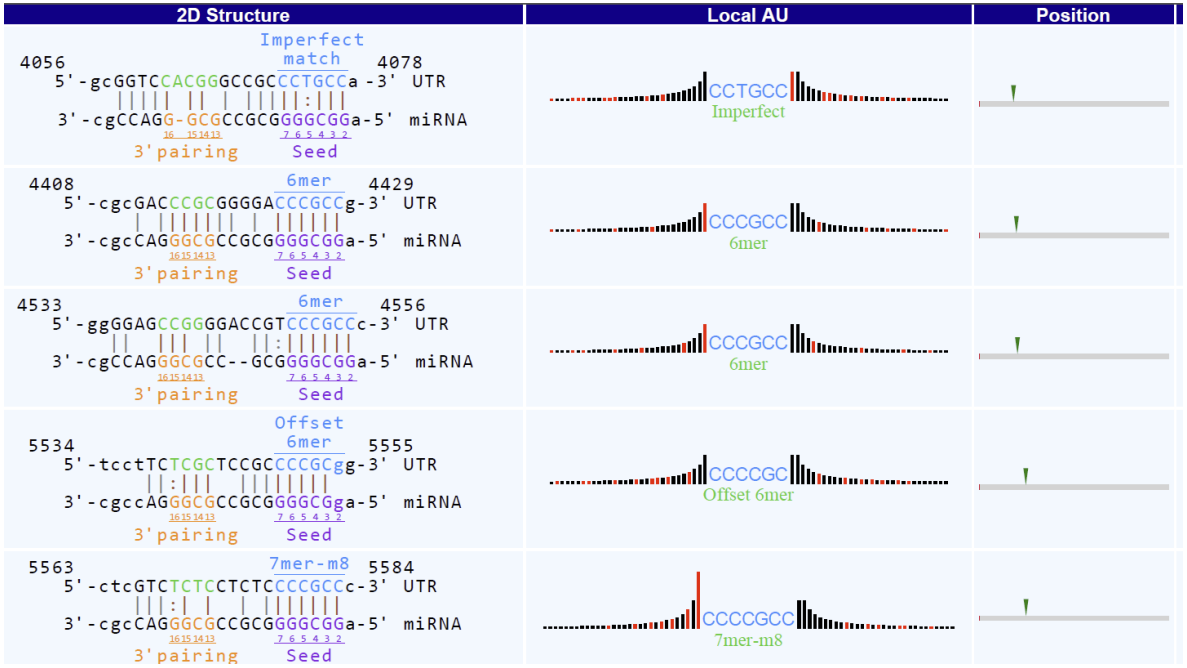


Fig. 4 Binding sites in hsa_circRNA_002143 for has-miR-663a. Only top 5 sites are listed here.

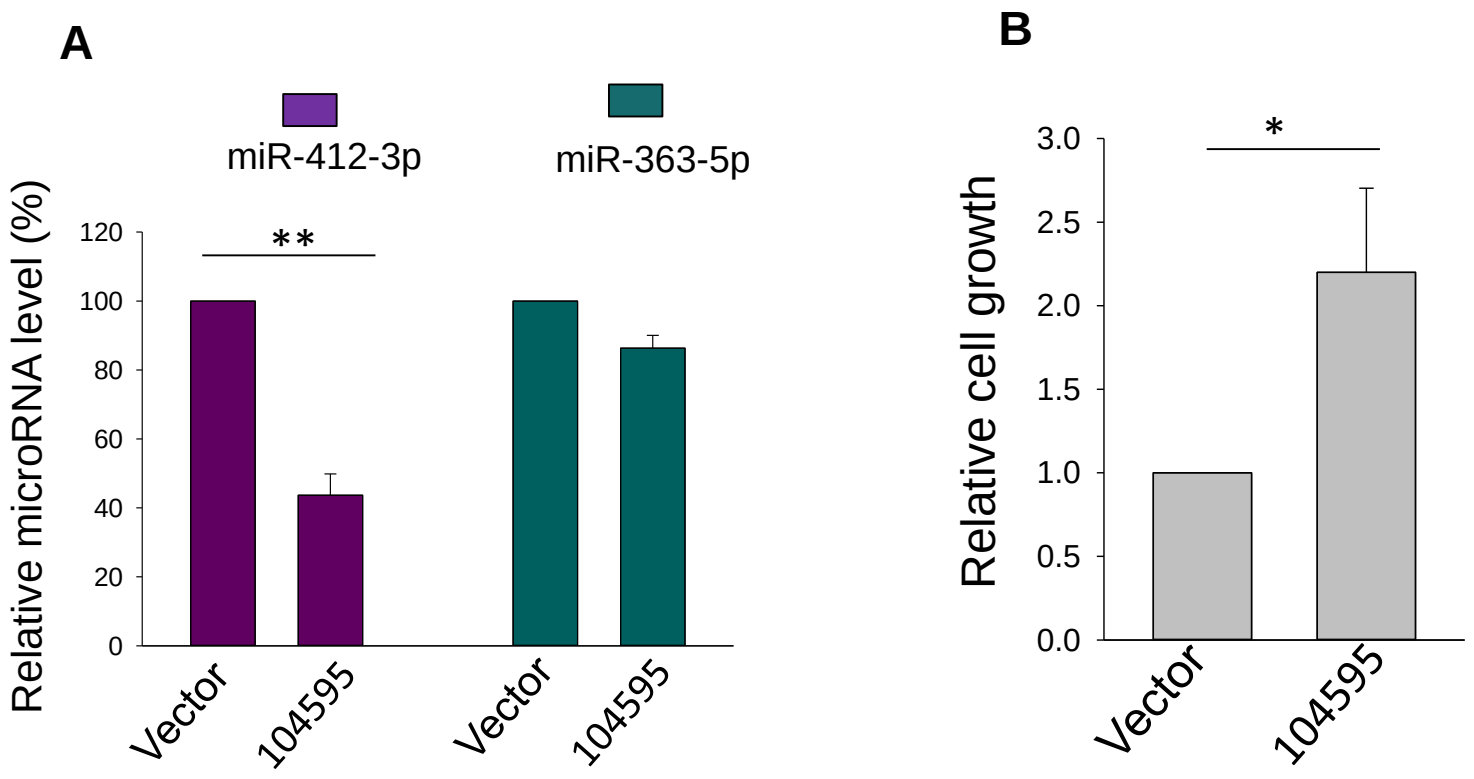


Fig. 5 Effect of overexpression of hsa_circRNA_104595 on microRNA expression and tumor cell growth. A, Hsa_circRNA_104595 downregulates miR-412-3p. LNCaP cells were transfected with hsa_circRNA_104595 or vector control. Total RNA was isolated for qRT-PCR analysis. B, Hsa_circRNA_104595 promotes tumor cell growth, as determined by MTT assay.

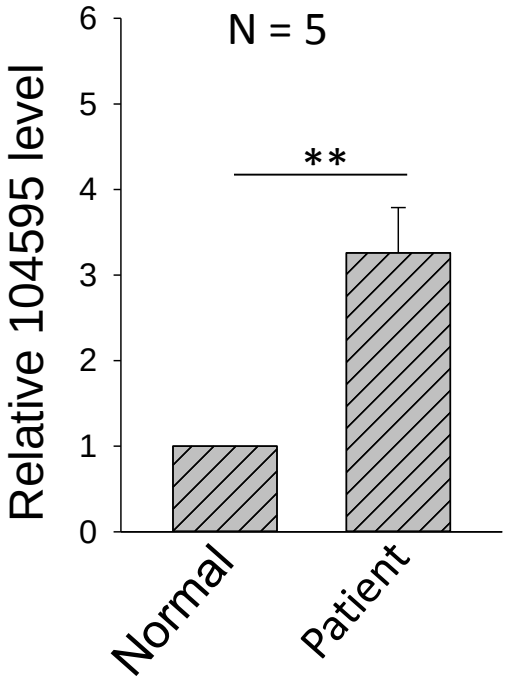


Fig. 6 Upregulation of hsa_circRNA_104595 in serum samples of prostate cancer patients, as detected by qRT-PCR.